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REPUBLIC OF SOUTH AFRICA



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Certificate

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DEPARTMENT OF TRADE AND  
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the documents attached hereto are true copies of the Forms P2, P6  
provisional specification and drawings of South African Patent Application  
No.2002/9830 in the names of University of Cape Town; South African  
Medical Research Council

Filed : 04 December 2002  
Entitled : A method for the production of HIV-1  
Subtype C PR55 GAG Virus-like  
particles

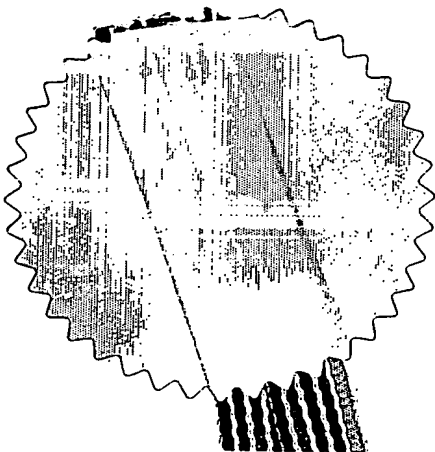
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12th January 2004  
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REPUBLIC OF SOUTH AFRICA  
PATENTS ACT, 1978

**APPLICATION FOR A PATENT**

AND ACKNOWLEDGEMENT OF RECEIPT  
(Section 30 (1) - Regulation 22)

REVENUE

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The granting of a patent is hereby requested by the undermentioned applicant on the basis of the present application filed in duplicate

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02002/9830		FULL NAME(S) OF APPLICANT(S)
71	1. UNIVERSITY OF CAPE TOWN 2. SOUTH AFRICAN MEDICAL RESEARCH COUNCIL	

ADDRESS(ES) OF APPLICANT(S)	
1. BREMNER BUILDING, LOVERS WALK, RONDEBOSCH, CAPE TOWN, SOUTH AFRICA 2. FRANCIE VAN ZIJL DRIVE, PAROW, 7505, WESTERN CAPE, SOUTH AFRICA	

TITLE OF INVENTION	
54	A METHOD FOR THE PRODUCTION OF HIV-1 SUBTYPE C PR55 GAG VIRUS-LIKE PARTICLES

THE APPLICANT CLAIMS PRIORITY AS SET OUT ON THE ACCOMPANYING FORM P.2. THE EARLIEST PRIORITY CLAIM IS:

COUNTRY: NIL	NUMBER: NIL	DATE: NIL
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THIS APPLICATION IS FOR A PATENT OF ADDITION TO PATENT APPLICATION NO.

21	01	
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THIS APPLICATION IS A FRESH APPLICATION IN TERMS OF SECTION 37 AND IS BASED ON APPLICATION NO.

21	01	
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THIS APPLICATION IS ACCOMPANIED BY:

- ☒ 1. A single copy of a provisional specification of 25 pages.
- ☒ 2. Drawings of 4 sheets.
- ☐ 3. Publication particulars and abstract (Form P.8 in duplicate).
- ☐ 4. A copy of Figure of the drawings (if any) for the abstract.
- ☐ 5. Assignment of invention.
- ☐ 6. Certified priority document.
- ☐ 7. Translation of the priority document.
- ☐ 8. Assignment of priority rights.
- ☐ 9. A copy of the Form P.2 and the specification of S.A. Patent Application No .
- ☐ 10. Declaration and power of attorney on Form P.3.
- ☐ 11. Request for ante-dating on Form P.4.
- ☐ 12. Request for classification on Form P.9.
- ☒ 13. Form P.2 in duplicate.
- ☐ 14. Other.

74	ADDRESS FOR SERVICE: SPOOR & FISHER, SANDTON
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Dated: 4 December 2002

*[Signature]*

SPOOR & FISHER  
PATENT ATTORNEYS FOR THE APPLICANT(S)

RECEIVED REGISTRAR OF PATENTS, DESIGNS, TRADE MARKS AND COPYRIGHT	
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REGISTRAR VAN PATENTE, MODELLE, HANDELSMERKE EN OUNTJES	

REPUBLIC OF SOUTH AFRICA			REGISTER OF PATENTS			PATENTS ACT, 1978			
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FULL NAME(S) OF INVENTOR(S)									
72	1. JAFFRAY, ANN 2. WILLIAMSON, ANNA-LISE 3. RYBICKI, EDWARD PETER								
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1. BREMNER BUILDING, LOVERS WALK, RONDEBOSCH, CAPE TOWN, SOUTH AFRICA 2. FRANCIE VAN ZIJL DRIVE, PAROW, 7505, WESTERN CAPE, SOUTH AFRICA									
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## PROVISIONAL SPECIFICATION

(Section 30(1) – Regulation 27)

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FULL NAMES OF APPLICANTS

71	UNIVERSITY OF CAPE TOWN SOUTH AFRICAN MEDICAL RESEARCH COUNCIL
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FULL NAMES OF INVENTORS

72	JAFFRAY, ANN WILLIAMSON, ANNA-LISE RYBICKI, EDWARD PETER
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TITLE OF INVENTION

54	A METHOD FOR THE PRODUCTION OF HIV-1 SUBTYPE C PR55 GAG VIRUS-LIKE PARTICLES
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## A METHOD FOR THE PRODUCTION OF HIV-1 SUBTYPE C PR55 GAG VIRUS-LIKE PARTICLES

### BACKGROUND OF THE INVENTION

THIS invention relates to a method for the production of HIV-1 subtype C Pr55 Gag virus-like particles, to the virus-like particles prepared by the method, and to the use of the virus-like particles in a vaccine.

The HIV genome contains three open reading frames. The *gag* open reading frame (Fig. 1) encodes a 55 kDa precursor protein (Pr55<sup>Gag</sup>) which is cleaved further by an HIV-encoded protease during virion maturation into three major structural proteins, a regulatory domain and 2 spacer peptides (Luciw, 1996). The structural proteins include the matrix (MA) protein (P17 – AA1 to AA132), the capsid (CA) protein (P24 – AA133 to AA363) and the nucleocapsid (NC) protein (P9 – AA377 to AA432). The regulatory domain (P6) spans AA449 to AA500 while the spacer regions P1 and P2 are ~~located~~ from AA433 to AA448 and AA364 to AA376 respectively (von Schwedler *et al.*, 1998).

The *pol* open reading frame overlaps that of *gag* from AA430 and is expressed via a ribosomal frame-shifting event that occurs at a frequency

of 5 to 10% during translation to produce a Gag-Pol precursor protein of 160kDa (Pr160) (Jacks *et al.*, 1988). The *pol* gene encodes several open reading frames including that for the protease, reverse transcriptase, RNase H and integrase enzymes of HIV-1. The *env* open reading frame lies further downstream of *pol* and encodes a 160 kDa precursor protein (gp160) of the viral envelope proteins gp41 and gp120 (Luciw, 1996).

After infection of a host cell, HIV-1 RNA is reverse transcribed into DNA which is subsequently integrated into the host genome (proviral stage). The Gag and Gag-Pol precursors are translated from transcribed HIV-1 provirus RNA in the cytosol and targeted to the host cell membrane. The Gag precursor associates with two copies of viral RNA and interacts with the Gag-Pol precursor to assemble into particle-like structures which line the host-cell membrane. They aggregate in such a way as to induce membrane curvature and subsequent bud formation during which viral Env proteins are also incorporated into the forming particles. The particles pinch off the membrane after which the HIV-1 particle maturation occurs, with the protease cleaving Gag and Gag-Pol into mature structural and functional proteins which lead to core condensation and thus a mature infectious virion.

Gag has been shown to assemble into virus-like particles (VLPs) in the absence of any other HIV-encoded genes in both mammalian and insect cells. These particles closely resemble the morphology of immature HIV virions and are non-infectious (Overton *et al.*, 1989; Gheysen *et al.*, 1989; Royer *et al.*, 1991; Royer *et al.*, 1992; Shioda and Shibuta, 1990; Vernon *et al.*, 1991; Mergener *et al.*, 1992). A number of Gag domains have been shown to be important in driving this particle assembly process and it has been shown that in fact about 80% of this precursor protein can be either deleted or replaced by heterologous sequences without significantly compromising VLP production (Accola *et al.*, 2000). These important domains are discussed below with respect to the functions of the individual proteins comprising Gag.

**MA protein (p17)**

The MA domain of Gag (Figure 2.) comprises a total of 132 amino acids and is responsible for targeting Gag precursor protein to the plasma membrane and virus-like particle assembly. The M domain (retrovirus membrane-binding domain) at the N-terminal of MA is mostly responsible for this function. MA has an N-terminal glycine residue which has been shown to be required for targeting Gag to the host cell membrane and facilitating particle assembly (Gheysen *et al.*, 1989). For this to occur, the glycine residue has to be myristylated. The amino acid recognition sequence for myristylation to occur at the N-terminus of Gag is gly-x-x-ser/thr.

The targeting and accumulation of HIV-1 Gag precursor at the host cell membrane by myristylation has been shown to occur in baculovirus-infected yeast cells, insect cells and mammalian cells (Jacobs *et al.*, 1989; Gheysen *et al.*, 1989; Bryant and Ratner, 1990). Substitution of the glycine residue eradicated particle formation, complementation of the residue restored VLP production and when using myr<sup>-</sup> mutants, Gag precursor was shown to accumulate in infected cell cytoplasm but did not associate with the host cell membrane. The myristyl moiety is thus required for stable membrane association of the particles. Only complete inhibition of Gag myristylation prevents VLP budding (Morikawa *et al.*, 1996), i.e. only a few myristylated Gag molecules are sufficient for plasma membrane targeting and budding.

Spearman (1997) has shown that myristate is the primary determinant of Gag-host cell membrane stability and therefore critical for particle assembly. Paillart and Göttlinger (1999) have proposed a model from results showing that the N-terminus of Gag is critical for insertion into the host cell membrane.

Apart from the myristylation signal other regions of MA have been shown to be important for targeting Gag to the host cell membrane and subsequent

particle assembly. Fäcke *et al.* (1993) showed that a large deletion of MA (AA16 to 99) caused drastic alteration of particle morphogenesis leading to immature particles produced in the endoplasmic reticulum instead. MA is required for the proper assembly of envelope proteins into the virion.

Yuan *et al.* (1993) showed that various MA deletions and substitutions caused a dramatic reduction in virus particle production. They demonstrated that it is possible that a polybasic region in MA (AA20 to 32) serves as part of a Gag transport signal to the membrane.

Zhou *et al.* (1994) studied this polybasic region further and showed that the highly basic residues form a positively charged surface which interacts with negatively charged phospholipids on the inner face of the lipid bilayer of the plasma. Ono and Freed (1999) have shown that a single mutation of MA (AA6 from V to R) severely impaired membrane binding without affecting myristylation.

#### Capsid (CA) protein (p24)

The CA domain (Figure 3.) encodes a protein of approximately 230 amino acids in length and has several domains which appear to be important for particle assembly, the first of which is a major homology region (MHR). The region extending from the N-terminus of CA downstream to the MHR is dispensable for particle formation, but any further deletions extending further into the MHR impair particle production (Borsetti *et al.*, 1998). Zhao *et al.*, (1994) also showed that baculovirus constructs of HIV-1 CA with a 10-amino acid deletion of AA140-150 as well as a separate deletion of AA250-260 led to the accumulation of viral protein at the cell membrane of insect cells. However there was no particle assembly or extracellular budding indicating that these two regions of CA at least, must play some role in normal particle formation.

On the other hand, Borsetti *et al.* (1998) showed that efficient particle formation occurred in the absence of both MA (excluding the myristyl anchor) and the N-terminal of the CA domain and therefore concluded that



there is no distinct region between the myristyl anchor and MHR which is absolutely essential for efficient particle release or assembly. They concluded that the C-terminal half of Gag contains protein-protein interaction domains which are essential for efficient particle assembly.

It seems that the C-terminal sequences may be required for protein-protein interactions but are not required for spherical particle formation and that the sphere is determined by the presence of an N-terminal extension on the CA domain.

The presence of RNA (heterogeneous in size and of viral and cellular origins) within the particles has been reported (Gheysen and Shioda and Shibuta)

Truncations into the P2 spacer regions have been shown to abolish particle formation.

#### **Spacer region 2 (p2)**

Borsetti *et al.*, 1998 have shown that the presence or absence of p2 determines the assembly of Gag proteins into spherical particles or cylindrical particles respectively. Morikawa *et al.* (2000) have also verified that this region is essential for VLP production in that if this region is truncated in any way, VLP production is abolished.

#### **Nucleocapsid (NC) protein (p7)**

The NC domain (Figure 4.) has been shown to contain two well-conserved Cys-His boxes resembling zinc finger motifs often found in DNA binding proteins. These are thought to play a role in RNA binding and encapsidation but influence some other aspects of particle assembly as well. There are two highly basic regions flanking these two motifs which have been shown to influence RNA binding *in vitro* and RNA encapsidation into virions if mutated. Jowett *et al.* (1992) showed that the deletion of the second Cys-His box did not affect particle formation but reduced RNA

binding substantially. However, they also showed that deletion of both Cys-His boxes encouraged the formation of larger particles and the loss of RNA binding altogether. The deletion of sequences upstream of the Cys-His boxes caused the abolition of particle-forming ability.

Dawson and Yu (1998) showed that the NC domain is essential for efficient assembly of HIV-1 and for the production of particles with wildtype density.

In addition to the Cys-His boxes, an I domain (interaction or assembly domain) close to the N-terminal of NC has been identified which is responsible for the formation of Gag protein complexes and also for the formation of punctate foci of Gag proteins at the plasma membrane. There are two positively charged basic arginine residues (AA380 and AA384) which have been shown to be critical for the function of the N-terminal I domain (interaction or assembly domain). Sandefur *et al.* (2000) have shown that I domain-deficient mutants block the formation of budding VLPs.

#### Spacer region 1 (p1)

CA and NC are separated by a short spacer region SP1 which is a protease cleavage site. Wieggers *et al.* (1998) have shown that when cleavage of SP1 from NC is prevented, maturation of particles is delayed and the ribonucleoprotein core has an irregular morphology. However, when SP1 cleavage from CA is prevented, normal condensation of the ribonucleoprotein core occurs but capsid condensation is prevented. They concluded from this that HIV maturation is a sequential process controlled by the rate of cleavage at individual sites.

#### P6 protein

Elements important for controlling particle size are contained within the C-terminal region of gag (P6) (Figure 5) as various deletions and substitutions of this region have been shown to induce the formation of very large particles (Garnier *et al.*, 1998). A specific domain referred to as the late (L) domain has been identified in P6 that is critical for the virus-cell separation

step. This region contains a PTAPP amino acid sequence. Sequences downstream of this domain in P6 were shown to be dispensable for virus release.

#### Other features of Gag influencing VLP formation

Buck *et al.* (2001) have recently found that mRNA of the HIV-1 Gag open reading frame exhibits internal ribosome entry site (IRES) activity that promotes translational initiation of Gag, producing a 40 kDa Gag protein. This IRES is located at an internal AUG codon found near the N-terminus of CA. This may have consequences when Gag is expressed in *in vitro* systems since initiation of translation is thought to be promoted by the direct binding of ribosomes with the participation of other host cell factors quite independently of the mRNA cap.

#### Minimal HIV-1 gag sequences required for VLP assembly and release

Although numerous domains of Gag have been shown to perform a particular function in Gag VLP assembly and release, deletion mutants of Gag have indicated that a number of these can be dispensable, and assembly of VLPs has been shown to be surprisingly tolerant to significant modifications of Gag protein (Wang *et al.*, 1998; Accola *et al.*, 2000; Wilk *et al.*, 2001). Wang *et al.*, (1998) made a series of C-terminal truncated mutants with which to examine VLP-producing capabilities. Truncated Gag precursors lacking most of the C-terminal Gag assembled into particles and were released from mammalian cells. A mutant with most of MA and the entire p6 region deleted still produced particles although less than wildtype particles. The smallest Gag product capable of VLP assembly was a 28 kDa protein which consisted of a few MA amino acids and the CA-p2 domain. The N-terminal portions of CA appeared to be critical when most of the MA domain was deleted, suggesting a requirement for an intact CA domain to assemble and release particles. Accola *et al.* (2000) showed that 80% of Gag could be deleted or replaced by heterologous sequences without significantly compromising VLP production. The smallest chimeric molecule still able to efficiently form VLPs was 16 kDa. This construct

contained a leucine zipper domain of the yeast transcription factor GCN4 to substitute for the assembly function of nucleocapsid, followed by a PPPPY motif to provide the L domain function, and retained only the myristylation signal and the C-terminal CA-p2 domain of Gag.

### **Ability of Gag to stimulate an immune response**

The long-term solution to combating the AIDS epidemic is through immunisation with a suitable vaccine and there are numerous HIV-1 genes and epitopes which are currently being used to develop vaccines. Although there is no Gag vaccine in use to date, a number of studies have shown that this protein induces an immune response in HIV-1-infected patients as well as in animals challenged with novel vaccines (Friedman *et al.*, 2000; Revskaya and Frankel, 2001). This response has been shown in some cases to be both humoral and cell-mediated (Qui *et al.*, 1999; Leung *et al.*, 2000; Qui *et al.*, 2000; Montefiori *et al.*, 2001; Kazanji *et al.*, 2001).

Goulder *et al.* (2000) did studies to identify the epitopes, which dominate the CTL response in ethnic groups and age groups worst hit by the global epidemic. They focussed on Gag because a number of gag specific responses have been shown to be associated with protection in HIV infection (Nixon and McMichael, 1991; Riviere *et al.*, 1995). The immunodominant Gag-specific CTL responses appeared to be focussed on 3 highly immunogenic regions, which together spanned 16% of the total length of Gag p17 and Gag p24 proteins but which represent two thirds of the dominant Gag-specific CTL responses detected. These results suggest that Gag would be an ideal candidate for vaccine design.

### **Plants as sources of vaccines**

There are many examples of the use of plants as sources of foreign protein and they are considered as viable and competitive expression systems for large-scale protein production (Doran, 2000). Favourable reasons for their use include the potential for large-scale, low-cost biomass production, a low risk of contamination by mammalian viruses and other animal pathogens,

the ability of plant cells to correctly fold and assemble multimeric proteins, and a low processing requirement for proteins administered orally in plant food or feed. They are thus considered a viable option for the production of foreign proteins, which can be used as vaccines.

A number of potentially useful vaccine candidates have been produced in plants and tested in animals. A useful technique for introducing foreign genes into plants has been via viral vector transmission.

Usha *et al.*, (1993) have used cowpea mosaic virus (CPMV) particles to express epitopes of foot and mouth disease virus (FMDV) on their coat protein as a result of a fusion on the coat protein gene.

Koo *et al.* (1999) made hybrids of tobacco mosaic virus (TMV) by fusing short epitopes from murine hepatitis virus (MHV) to TMV coat protein and subsequently propagating them in tobacco plants.

Fernández-Fernández *et al.* (2001) have developed a plum pox potyvirus vector for the expression of foreign proteins. They have used it to express an antigenic structural protein of rabbit hemorrhagic disease virus (RHDV), producing chimeric virus particles which when inoculated into rabbits produce an immune response against RHDV.

A number of other methods have been used to produce vaccine candidates in plants. McCormick *et al.* (1999) demonstrate the modification of a TMV vector such that it not only produces single chain Fv fragments in plants, but secretes them into the apoplast. This makes harvesting of the product a lot simpler than having to isolate foreign proteins from leaf extracts. Several attempts have also been made to make plants transgenic for production of foreign proteins to be used as vaccine candidates. Mason *et al.* (1996) have made transgenic tobacco and potato plants to successfully produce Norwalk virus capsid protein, which shows immunogenicity in mice. Wigdorowitz *et al.* (1999) have made transgenic alfalfa plants expressing an antigenic protein against FMDV and shown that animals immunised with purified antigen show immunogenicity against the virus.

There have been a few attempts at producing candidate vaccines against HIV-1 in plants. Yusibov *et al.* (1997) have used the coat protein of alfalfa mosaic virus as a carrier molecule to express antigenic peptides from HIV-1 (V3 loop). *In vitro* transcripts of recombinant virus with sequences encoding the antigenic peptides were synthesised from DNA constructs and used to inoculate tobacco plants. Recombinant virus particles were produced and purified and used for immunisation of mice. The antigens elicited specific virus-neutralizing antibodies in immunised mice. Zhang *et al.* (2000) used a tomato bushy stunt virus (TBSV) as an expression vector of HIV-1 p24 protein. This gene was introduced into the TBSV genome as an in-frame fusion with a 5' terminal portion of the TBSV coat protein ORF. Introduction into plants led to the accumulation of p24 fusion proteins in inoculated leaves.

#### **SUMMARY OF THE INVENTION**

According to a first embodiment of the invention, there is provided a method of producing an HIV-1 subtype C immunogenic protein or a related amino acid sequence, the method comprising the steps of:

introducing into a plant a tobacco mosaic virus vector or vector system including a nucleic acid sequence encoding the HIV-1 subtype C immunogenic protein or related amino acid sequence derived by substitution, deletion and/or insertion of one or more nucleotides, and/or extension or truncation of one or both ends thereof;

causing expression of the nucleic acid sequence in the plant; and

causing expression of the nucleic acid sequence in the plant; and

recovering the resulting HIV-1 subtype C immunogenic protein or related amino acid sequence produced within the plant.

The nucleic acid sequence preferably hybridises, under standard conditions, to at least a part of an isolated nucleic acid sequence coding for the immunogenic protein, or the complement of the nucleic acid sequence coding for an immunogenic protein or to its degenerate equivalents.

The immunogenic protein may be HIV-1 subtype C Pr55 Gag. In particular, the protein may be assembled into the form of virus-like particles (VLPs).

The nucleic acid sequence may be selected so as to cause or aid assembly of the immunogenic protein into virus-like particles.

The nucleic acid sequence may be at least part of the *gag* gene obtained from HIV-1 isolate DU422, assigned provisional accession no. 01032114 by the European Collection of Cell Cultures.

The plant may be a tobacco plant.

According to a second embodiment of the invention, there are provided HIV-1 subtype C immunogenic proteins or related amino acid sequences obtained from the method substantially as described above. The protein may be HIV-1 subtype C Pr55 Gag, and may be assembled into the form of virus-like particles (VLPs).

According to a further embodiment of the invention, there is provided a vector or vector system which expresses HIV-1 subtype C Pr55 Gag in tobacco plants.

The vector may be a tobacco mosaic virus vector.

According to yet a further embodiment of the invention, there is provided a recombinant vector system including a tobacco mosaic virus vector containing a nucleic acid sequence encoding an HIV-1 subtype C immunogenic protein or a related amino acid sequence derived by substitution, deletion and/or insertion of one or more nucleotides, and/or extension or truncation of one or both ends thereof; said nucleic acid

sequence hybridising, under standard conditions, to at least a part of an isolated nucleic acid sequence coding for the immunogenic protein, or the complement of the nucleic acid sequence coding for an immunogenic protein or to its degenerate equivalents.

The immunogenic protein may be HIV-1 subtype C Pr55 Gag.

The nucleic acid sequence may be selected to cause or aid assembly of the immunogenic protein into virus-like particles.

According to yet a further embodiment of the invention, there is provided a host cell including the vector or vector system substantially as described above.

The host cell is preferably a plant cell, and may be a tobacco plant cell.

According to yet a further embodiment of the invention, there is provided a vaccine for use in the treatment or prophylaxis of HIV infection in a mammal, the vaccine including virus-like particles produced substantially according to the method described above.

Preferably, the vaccine will be sufficient to induce an immunogenic response to the virus-like particles in a suitable susceptible host.

The vaccine may include a pharmaceutical excipient and/or adjuvant, and a therapeutically effective amount of the virus-like particles.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1** shows a schematic representation of the *gag* open reading frame of the HIV-1 genome;

**Figure 2** shows a schematic representation of the matrix (MA) protein (p24) domain of the *gag* gene of Figure 1;



- Figure 3** shows a schematic representation of the capsid (CA) protein (p24) domain of the *gag* gene of Figure 1;
- Figure 4** shows a schematic representation of the nucleocapsid (NC) protein (p7) domain of the *gag* gene of Figure 1;
- Figure 5** shows a schematic representation of the domain of the p6 protein domain of the *gag* gene of Figure 1;
- Figure 6** shows the DNA sequence of the DU422 *gag* sequence used for cloning into pBSG1057. The bold underlining represents the *gag* gene and the dotted underlining represents the partial *pol* fragment;
- Figure 7** shows a plasmid map of pBSG1057;
- Figure 8** shows a plasmid map of pBSGgag6; and
- Figure 9** shows a plasmid map of pBSGgagopt11.

#### **DETAILED DESCRIPTION OF AN EMBODIMENT OF THE INVENTION**

The invention will now be described in more detail with reference to a particular embodiment of the invention.

#### **Cloning of *Gag* into TMV vector pBSG1057**

The *Gag* gene was obtained from HIV-1 isolate DU422 (obtained from a ~~South~~ African sex worker cohort, and assigned provisional accession no. 01032114 by the European Collection of Cell Cultures) (Figure 6). It comprises of the entire *gag* gene and the first 57 bases of the *pol* gene. It was cloned into the *Eco*RI and *Sal*I restriction enzyme sites of an *E. coli* ~~vector~~ pGEM-T easy™. The ends of the gene were modified by PCR amplification such that *Pac*I and *Xho*I restriction enzyme sites were

attached to the 5' and 3' ends respectively, to facilitate cloning into the TMV vector pBSG1057 (Large Scale Biology Corporation) (Figure 7). Amplification products were re-cloned into pGEM-T easy™ and sequenced to verify the integrity of the restriction enzyme sites and the *gag* sequence.

The green fluorescent protein (GFP) gene sequence was excised from pBSG1057 by restriction enzyme digestion with *PacI* and *XhoI*, and *gag* cloned into the TMV vector at these 2 sites to produce the clone pBSGgag6 (Figure 8).

#### **Codon-optimisation of *gag* gene for *Nicotiana***

An additional strategy was undertaken to codon-optimize the *gag* gene for *Nicotiana*, synthesise it, and clone it using a similar strategy to that described above into the pBSG1057 TMV vector with the hope that this gene would enhance Gag protein expression when introduced into *N. benthamiana*. The codon-optimized *gag* gene was cloned into the *PacI* and *XhoI* restriction enzyme sites of pBSG1057 after removal of GFP. The resultant clone was called pBSGgagopt11 (Figure 9).

#### **Transcription of pBSGgag6, pBSGgagopt11 and pBSG1057**

mRNA of pBSGgag6, pBSGgagopt11 and pBSG1057 was produced using a Ribomax Transcription/translation kit (Promega). Ten micrograms of each plasmid was used per reaction.

#### **Infection of *Nicotiana benthamiana* with recombinant TMV mRNA**

The mRNA (50µl) was rubbed over an expanding leaf of 6-week old *N. benthamiana* plants using cotton-wool buds. Plants inoculated with the control pBSG1057 mRNA were monitored daily with a UV light for the appearance of GFP in both inoculated and upper leaves of the plants. Systemic spread of GFP was used as an indicator of systemic spread of recombinant TMV and leaves were sampled for detection of Gag protein by western blotting, EM analysis and ELISA.

### Detection of Gag protein

Crude protein preparations were made by crushing up leaves in a mortar and pestle, filtration of remaining solid matter through cheesecloth, and addition of loading buffer.

### Western blotting

The samples were boiled for 5 minutes and run on 10% SDS polyacrylamide gels to separate the proteins. Resolved proteins were transblotted from the SDS polyacrylamide gels onto nitrocellulose membranes. Membranes were probed with an anti-mouse p17 monoclonal antibody (Chemicon) at a dilution of 1:200.

### EM analysis for Gag VLPs

Crude leaf extracts were centrifuged at low speed to pellet remaining unground tissue. Small amounts of leaf extract were dried on copper grids and immunotrapped with an anti-mouse p17 monoclonal antibody (Chemicon) at a dilution of 1:200. The grids were counterstained with uranyl acetate and looked at using a transmission electron microscope.

### ELISA

Crude leaf extracts were centrifuged at low speed to pellet remaining unground tissue. Small amounts were diluted in 1 X PBS and used for detection of p24 antigen by using the Vironostika® HIV-1 antigen ELISA kit.

## RESULTS

### Baculovirus expression of HIV-1 subtype C gag VLPs

HIV-1 subtype C Gag VLPs were produced using the Bac-to-Bac® baculovirus expression system (Life Technologies). These provide a

relevant positive control for further protein (Gag) detection experiments and can be used to generate antibodies specific to HIV-1 subtype C Gag.

Because the cloned *gag* gene contains an N-terminal glycine residue sequence, it is expected that the recombinant protein be targeted to the host plasma membrane. VLPs thus formed were subsequently budded from the cell surface into the insect cell medium. VLPs were purified from the insect cell medium following a sucrose density gradient protocol for baculovirus-derived HIV VLPs detailed by Nermut *et al* (1994). The resulting protein band after centrifugation on the density gradient was extracted, dialysed in phosphate-buffered saline (PBS pH 7.4) overnight and small amounts used for SDS Page analysis, western blotting and EM analysis. VLPs of approximately 110 to 120 nm in diameter were visualized under the electron microscope, verifying successful *gag* VLP production. A single 55kD protein band was visualized in samples resolved on an SDS page gel, and a monoclonal and a polyclonal antibody to *gag* P17 protein were found to react positively using western blot analysis. Two additional monoclonal antibodies to *gag* P24 protein were tested subsequently against baculovirus-derived VLPs and reacted positively using western blot analysis.

#### **Assaying for expression of *gag* in *N. benthamiana***

*N. benthamiana* plants were inoculated with mRNA transcripts of the pBSGgag6 and pBSGgagopt11 clones as well as with the TMV vector containing GFP (pBSG1057) as described previously. Water-inoculated plants served as negative controls. Plants were grown under normal growth conditions in plant rooms and monitored most days for the appearance of green fluorescent spots in the control plants inoculated with pBSG1057 mRNA transcripts, as well as for TMV symptoms in pBSGgag6-, pBSGgagopt11- and pBSG1057-inoculated plants. Leaves were periodically sampled for EM analysis as well as western blot analysis and ELISA on crude extracts.

TMV symptoms and GFP fluorescence:

Green fluorescent spots were visible under the UV light on the inoculated leaves of those infected with pBSG1057 mRNA transcript at 4 days post inoculation (dpi). Spread of the GFP spots to upper leaves was visible at 10 dpi. TMV symptoms were visible in the newer growth of pBSG1057-inoculated plants at 17 dpi and in the pBSGgag6- and pBSGgagopt11-inoculated plants at 24 dpi.

Western blotting

Lanes containing crude protein preparations from pBSGgag6- and pBSGgagopt11-inoculated leaves did not yield any positive result compared with baculovirus-produced gag which highlighted the presence of a 55kD protein.

EM analysis (immunotrapping):

EM analysis was performed using crude apical leaf extracts (35 dpi) ground up in PBS (pH 7.4) and trapped with a concentrated solution of p17 monoclonal antibodies. There was very little plant material present in the pBSG1057-inoculated sample (no particles ranging from 100 to 120 nm in size) and very few TMV particles. In the pBSGgag6-inoculated sample there were a large number of 100 to 110 nm-sized VLPs as well as quite a few TMV subunit-sized particles (25 nm). In the pBSGgagopt11-inoculated sample, there were similar shaped but less 100 to 110 nm-sized particles to those seen in the pBSGgag6-inoculated sample.

ELISA

Preliminary results of ELISA performed on crude preparations of protein showed the presence of gag protein in pBSGgagopt11-inoculated leaves at a level of 460pg/ml crude preparation protein solution.

Although the invention has been described above with reference to one particular embodiment, it is not intended that this should limit the invention to what has been described above.

#### REFERENCES

Accola, M. A., Strack, B. and Göttlinger, H. G. 2000. Efficient particle production by minimal Gag constructs which retain the carboxy-terminal domain of human immunodeficiency virus type 1 capsid-p2 and a late assembly domain. *J. Virol.* **74**: 5395-5402.

Borsetti, A., Öhagen, Å. and Göttlinger, H. G. 1998. The C-terminal half of the human immunodeficiency virus type 1 gag precursor is sufficient for efficient particle assembly. *J. Virol.* **72**: 9313-9317.

Buck, C. B., Shen, X., Egan, M. A., Pierson, T. C., Walker, C. M. and Siliciano, R. F. 2001. The human immunodeficiency virus type 1 *gag* encodes an internal ribosome entry site. *J. Virol.* **75**: 181-191.

Bryant, M. and Ratner, L. 1990. Myristolation-dependent replication and assembly of human immunodeficiency virus 1. *Proc. Natl. Acad. Sci. USA* **87**: 523-527.

Dalsgaard, K., Uttenthal, Å., Jones, T. D., Xu, F., Merryweather, An., Hamilton, W. D. O., Langeveld J. P. M., Boshuizen, R. S., Kamstrup, S., Lomonossoff, G. P., Porta, C., Vela, C., Casal, J. I., Meloen, R. H. and Rodgers P. B. 1997. Plant-derived vaccine protects target animals against a viral disease. *Nature Biotech.* **15**: 248-252.

Dawson, L. and Yu, X-F. 1998. The role of the nucleocapsid of HIV-1 in virus assembly. *Virology* **251**: 141-157.

Doran, P. M. 2000. Foreign protein production in plant tissue cultures. *Curr. Opin. Biotech.* **11**: 199-204.

Fäcke, M., Janetzko, A., Shoeman, R. L. and Kräusslich, H-G. 1993. A large deletion in the matrix domain of the human immunodeficiency virus *gag* gene redirects virus particle assembly from the plasma membrane to the endoplasmic reticulum. *J. Virol.* **67**: 4972-4980.

Fernández-Fernández, M. R., Mourino, M., Rivera, J., Rodríguez, F., Plana-Durán, J. and García, J. A. 2001. Protection of rabbits against rabbit hemorrhagic disease virus by immunization with the VP60 protein expressed in plants with a potyvirus-based vector. *Virology* **280**: 283-291.

Forster, M. J., Mulloy, B. and Nermut, M. V. 2000. Molecular modelling study of HIV p17gag (MA) protein shell utilising data from electron microscopy and X-ray crystallography. *J. Mol. Biol.* **298**: 841-857.

Freed, E. O. 1998. HIV-1 Gag proteins: diverse functions in the virus life cycle. *Virology* **251**: 1-15.

Friedman, R. S., Frankel, F. R., Xu, Z. and Lieberman, J. 2000. Induction of human immunodeficiency virus (HIV)-specific CD8 T-cell responses by *Listeria monocytogenes* and a hyperattenuated *Listeria* strain engineered to express HIV antigens. *J. Virol.* **74**: 9987-9993.

Fuller, S. D., Wilk, T., Gowen, G. E., Kräusslich, H-G. and Vogt, V. M. 1997. Cryo-electron microscopy reveals ordered domains in the immature HIV-1 particle. *Curr. Biol.* **7**: 729-738.

Garnier, L., Ratner, L., Rovinski, B., Cao, S. X. and Wills, J. W. 1998. Particle size determinants in the human immunodeficiency virus type 1 gag protein. *J. Virol.* **72**: 4667-4677.

Gheysen, D., Jacobs, E., de Forests, F., Thiriart, C., Francotte, M., Thines, D. and De Wilde, M. 1989. Assembly and release of HIV-1 precursor Pr55<sup>Gag</sup> virus-like particles from recombinant baculovirus-infected insect cells. *Cell* **59**: 103-112.

Goulder, P. J. R., Brander, C., Annamalai, K., Mngqundaniso, N., Govender, U., Yang, Y., He, S., Hartmen, K. E., O'Callaghan, C. A., Ogg, G. S., Altfeld, M. A., Rosenberg, E. S., Cao, H., Kalams, S. A., Hammond, M., Bunce, M., Pelton, S. I., ~~Durheim~~ S., A., McIntosh, K., Coovadia, H. M. and Walker, B. D. 2000. ~~Differential~~ narrow focusing of immunodominant human immunodeficiency virus gag-specific cytotoxic T-lymphocyte responses in infected african and caucasoid adults and children. *J. Virol.* **74**: 5679-5690.

Gross, I., Hohenberg, H., Huckhagel, C. and Kräusslich, H-G. 1998. N-terminal extension of human immunodeficiency virus capsid proteins converts the *in vitro* assembly phenotype from tubular to spherical particles. J. Virol. **72**: 4798-4810.

Hill, C. P., Worthylake, D., Bancroft, D. P., Christensen, A. M. and Sundquist, W. I. 1996. Crystal structures of the trimeric human immunodeficiency virus type 1 matrix protein: implications for membrane association and assembly. Proc. Natl. Acad. Sci. USA **93**: 3099-3104.

Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J. and Varmus, H. E. 1988. Characterisation of ribosomal frameshifting in HIV-1 gag-pol expression. Nature **331**: 280-283.

Jacobs, E., Gheysen, D., Thines, D., Francotte, M. and de Wilde, M. 1989. The HIV-1 Gag precursor Pr55<sup>gag</sup> synthesized in yeast is myristoylated and targeted to the plasma membrane. Gene **79**: 71-81.

Jowett, J. B. M., Hockley, D. J., Nermut, M. V. and Jones, I. M. 1992. Distinct signals in human immunodeficiency virus type 1 Pr55 necessary for RNA binding and particle formation. J. Gen. Virol. **73**: 3079-3086.

Kazanji, M., Tartaglia, J., Franchini, G., de Thoisy, B., Talarmin, A., Contamin, H., Gessain, A. and de Thé, G. 2001. Immunogenicity and protective efficacy of recombinant human T-cell leukemia/lymphoma virus type 1 NYVAC and naked DNA vaccine candidates in squirrel monkeys (*Saimiri sciureus*). J. Virol. **75**: 5939-5948.

Koo, M., Bendahmane, M., Lettieri, G. A., Paoletti, A. D., Lane, T. E., Fitch, J. H., Buchmeier, M. J. and Beachy, R. N. 1999. Protective immunity against murine hepatitis virus (MHV) induced by intranasal or subcutaneous administration of hybrids of tobacco mosaic virus that carries an MHV epitope. Proc. Natl. Acad. Sci. USA **96**: 7774-7779.

Leung, N. J., Aldovini, A., Young, R., Jarvis, M. A., Smith, J. M., Meyer, D., Anderson, D. E., Carlos, M. P., Gardner, M. B. and Torres, J. V. 2000. The kinetics of specific immune responses in rhesus monkeys inoculated with live recombinant BCG expressing SIV gag, pol, env, and nef proteins. Virology **268**: 94-103.



Luciw, P. 1996. Human immunodeficiency viruses and their replication. In *Fields Virology* (Fields, B. N., Knipe, D. M., Howley, eds) pp1881-1952, Lippincott - Raven Publishers, Philadelphia.

Mason, H. S., Ball, J. M., Shi, J.-J., Jiang, X., Estes, M. K. and Arntzen, C. J. 1996. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proc. Natl. Acad. Sci. USA* **93**: 5335-5340.

McCormick, A. A., Kumagai, M. H., Hanley, K., Turpen, T. H., Hakim, I., Grill, L. K., Tusé, D., Levy, S. and Levy, R. 1999. Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants. *Proc. Natl. Acad. Sci. USA* **96**: 703-708.

Mergener, K., Fäcke, M., Welker, R., Brinkmann, V., Gelderblom, H. R. and Kräusslich, H.-G. 1992. Analysis of HIV particle formation using transient expression of subviral constructs in mammalian cells. *Virology* **186**: 25-39.

Morikawa, Y., Hinata, S., Tomoda, H., Goto, T., Nakai, M., Aizawa, C., Tanaka, H. and Omura, S. 1996. Complete inhibition of human immunodeficiency virus gag myristoylation is necessary for inhibition of particle budding. *J. Biol. Chem.* **271**: 2868-2873.

Montefiori, D. C., Safrit, J. T., Lydy, S. L., Barry, A. P., Bilska, M., Vo, H. T. T., Klein, M., Tartaglia, J., Robinson, H. L. and Rovinski, B. 2001. Induction of neutralizing antibodies and gag-specific cellular immune responses to an R5 primary isolate of human immunodeficiency virus type 1 in rhesus macaques. *J. Virol.* **75**: 5879-5890.

Morikawa, Y., Hockley, D. J., Nermut, M. V. and Jones, I. M. 2000. Roles of matrix, p2 and N-terminal myristoylation in human immunodeficiency virus type 1 gag assembly. *J. Virol.* **74**: 16-23.

Nermut, M. V., Hockley, D. J., Jowett, J. B. M., Jones, I. M., Garreau, M. and Thomas, D. 1994. Fullerene-like organization of HIV gag-protein shell in virus-like particles produced by recombinant baculovirus. *Virology* **198**: 288-296.

Nixon, D. F. and McMichael, A. J. 1991. Cytotoxic T cell recognition of HIV proteins and peptides. *AIDS* **5**: 1049-1059.

Ono, A. and Freed, E. O. 1999. Binding of human immunodeficiency virus type 1 Gag to membrane: rôle of the matrix amino terminus. *J. Virol.* **73**: 4136-4144.

Overton, H.A., Fujii, Y., Price, I. R. and Jones I. M. 1989. The protease and gag gene products of the human immunodeficiency virus: authentic cleavage and post-translational modification in an insect cell expression system. *Virology* **170**: 107-116.

Paillart, J-C. and Göttlinger, H. G. 1999. Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of gag membrane targeting. *J. Virol.* **73**: 2604-2612.

Qui, J-T., Song, R., Dettenhofer, M., Tian, Ch., August, T., Felber, B. K., Pavlakis, G. N. and Yu, X-F. 1999. Evaluation of novel human immunodeficiency virus type 1 gag DNA vaccines for protein expression in mammalian cells and induction of immune responses. *J. Virol.* **73**: 9145-9152.

Qui, J-T., Liu, B., Tian, C., Pavlakis, G. N. and Yu, X-F. 2000. Enhancement of primary and secondary cellular immune responses against human immunodeficiency virus type 1 gag by using DNA expression vectors that target gag antigen to the secretory pathway. *J. Virol.* **74**: 5997-6005.

Revskeya, M. V. and Frankel, F. R. 2001. Systemic immunity and mucosal immunity are induced against human immunodeficiency virus gag protein in mice by a new hyperattenuated strain of *Listeria monocytogenes*. *J. Virol.* **75**: 2786-2791.

Riviere, Y., McChesney, M. B., Porrot, F., Tanneau-Salvadori, F., Sansonetti, P., Lopez, O., Pialoux, G., Feuillie, V., Mollereau, M. and Chamaret, S. 1995. Gag-specific cytotoxic responses to HIV type 1 are associated with a decreased risk of progression to AIDS-related complex or AIDS. *AIDS Res. Hum. Retrovir.* **11**: 903-907.

Royer, M., Cerutti, M., Gray, B., Hong, S-S., Devauchelle, G. and Boulanger, P. 1991. Functional domain of HIV-1 gag polyprotein expressed in baculovirus-infected cells. *Virology* **184**:417-422.

Royer, M., Hong, S.-S., Gay, B., Cerutti, M. and Boulanger, P. 1992. Expression and extracellular release of human immunodeficiency virus type 1 Gag precursors by recombinant baculovirus-infected cells. *J. Virol.* **66**:3230-3235.

Sandefur, S., Smith, R. M., Varthakavi, V. and Spearman, P. 2000. Mapping and characterization of the N-terminal I domain of human immunodeficiency virus type 1 p55. *J. Virol.* **74**: 7238-7249.

Shehu-Xhilaga, M., Crowe, S. M. and Mak, J. 2001. Maintenance of the Gag/Gag-Pol ratio is important for human immunodeficiency virus type 1 RNA dimerization and viral infectivity. *J. Virol.* **75**: 1834-1841.

Shioda, T. and Shibuta, H. 1990. Production of human immunodeficiency virus (HIV)-like particles from cells infected with recombinant vaccinia viruses carrying the gag gene of HIV. *Virology* **175**: 139-148.

Spearman, P., Horton, R., Ratner, L. and Kuli-Zade, I. 1997. Membrane binding of human immunodeficiency virus type 1 matrix protein in vivo supports a conformational myristyl switch mechanism. *J. Virol.* **71**: 6582-6592.

Usha, R., Rohll, J. B., Spall, V. E., Shanks, M., Maule, A. J., Johnson, J. E. and Lonomosoff G. P. 1993. Expression of an animal virus antigenic site on the surface of a plant virus particle. *Virology* **197**: 366-374.

Vernon, S. K., Murthy, S., Wilhelm, J., Chanda, P. K., Kalyan, N., Lee, S.-G. and Hung, P. P. 1991. Ultrastructural characterisation of human immunodeficiency virus type 1 Gag-containing particles assembled in a recombinant adenovirus vector system. *J. Gen. Virol.* **72**: 1243-1251.

von Schwedler, U. K., Stemmler, T. L., Klishko, V. Y., Li, S., Albertine, K. H., Davis, D. R. and Sundquist, W. I. 1998. Proteolytic refolding of the HIV-1 capsid protein amino-terminus facilitates viral core assembly. *EMBO J.* **17**: 1555-1568.

Wang, C.-T., Lai, H.-Y. and Li, J.-J. 1998. Analysis of minimal human immunodeficiency virus type 1 gag coding sequences capable of virus-like particle assembly and release. *J. Virol.* **72**: 7950-7959.

Wiegers, K., Rutter, G., Kottler, H., Tessmer, U., Hohenberg, H. and Kräusslich, H.-G. 1998. Sequential steps in human immunodeficiency virus particle maturation

revealed by alterations of individual gag polyprotein cleavage sites. J. Virol. 72: 2846-2854.

Wigdorivitz, A., Carrillo, C., Dus Santos, M. J., Trono, K., Peralta, A., Gómez, M. C., Ríos, R. D., Franzone, P. M., Sadir, A. M., Escribano, J. M. and Borca, M. V. 1999. Induction of a protective antibody response to foot and mouth disease virus in mice following oral or parenteral immunization with alfalfa transgenic plants expressing the viral structural protein VP1. Virology 255: 347-353.

Wilk, T., Gross, I., Gowen, B. E., Rutten, T., de Haas, F., Welker, R., Kräusslich, H-G., Boulanger, P. and Fuller, S. D. 2001. Organisation of immature human immunodeficiency virus type 1. J. Virol. 75: 759-771.

Yuan, X., Yu, X., Lee, T-H. and Essex, M. 1993. Mutations in the N-terminal regions of human immunodeficiency virus type 1 matrix protein block intracellular transport of the gag precursor. J. Virol. 67: 6387-6394.

Yusibov, V., Modelska, N., Steplewski, K., Agadjanyan, M., Weiner, D., Hooper, D. C. and Koprowski, H. 1997. Antigens produced in plants by infection with chimeric plant viruses immunize against rabies virus and HIV-1. Proc. Natl. Acad. Sci. USA 94: 5784-5788.

Zhang, G., Leung, C., Murdin, L, Rovinski, B. and White, K. A. 2000. *In planta* expression of HIV-1 p24 protein using an RNA plant virus-based expression vector. Mol Biotechnol. 14: 99-107.


Zhao, Y., Jones, I. M., Hockley, D. J., Nermut, M., V. and Roy, P. 1994. Complementation of human immunodeficiency virus (HIV-1) gag particle formation. Virology 199: 403-408.

Zhou, W., Parent, L. J., Wills, J. W. and Resh, M. D. 1994. Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 gag protein which interacts with acidic phospholipids. J. Virol. 68: 2556-2569.

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DATED THIS 4<sup>TH</sup> DAY OF NOVEMBER 2002



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SPOOR & FISHER  
APPLICANT'S PATENT ATTORNEYS

Gag (Pr55<sup>gag</sup>) polyprotein precursor

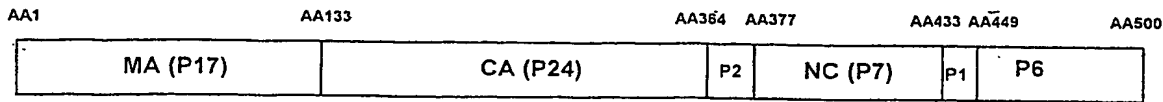


Figure 1

Matrix (MA) protein

Comprises the 3D globular domain of MA

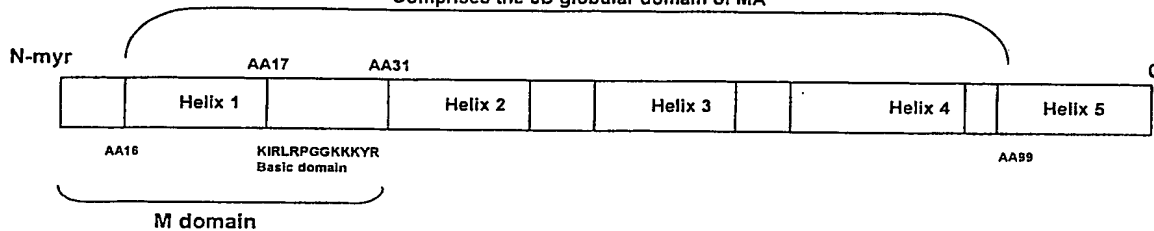


Figure 2

Nucleocapsid (NC) protein (p7)

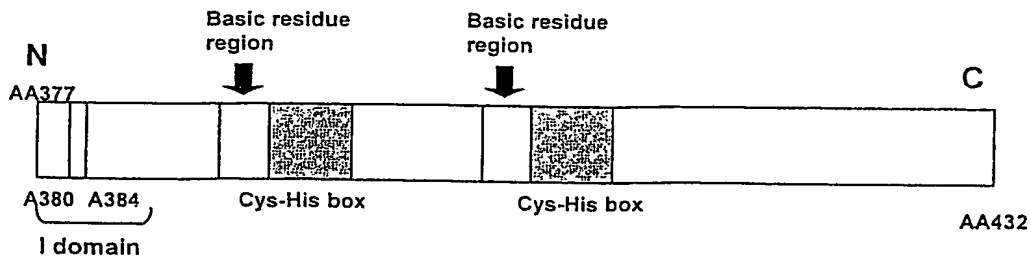


Figure 3

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Capsid (CA) protein (p24)

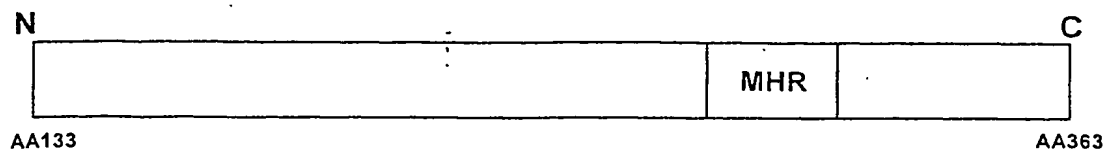


Figure 4

P6 protein

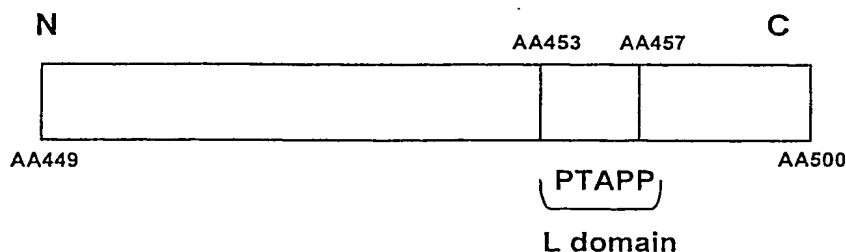


Figure 5

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Figure 6

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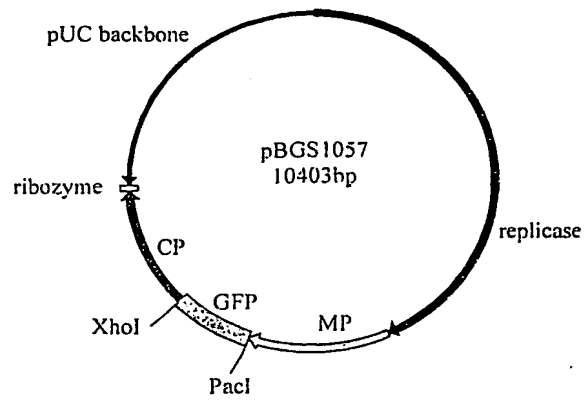


Figure 7

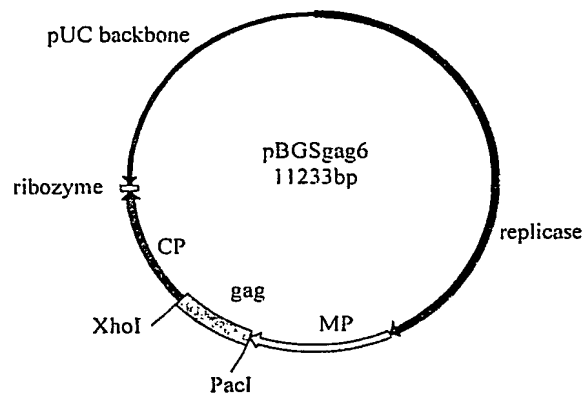


Figure 8



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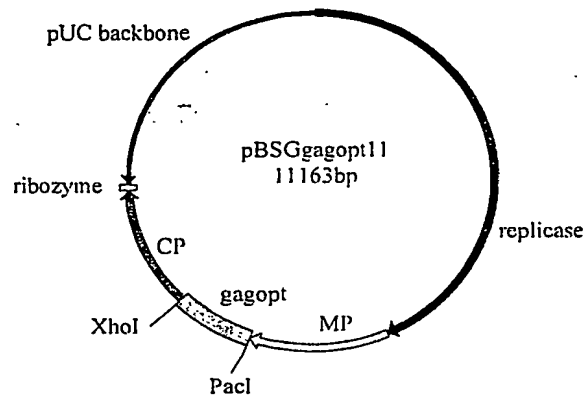


Figure 9